ORIGINAL RESEARCH ARTICLE

Clin Drug Investig 2010; 30 (1): 5-14
1173-2563/10/0001-0005/$49.95/0



# Effects of Prulifloxacin on Cardiac Repolarization in Healthy Subjects

## A Randomized, Crossover, Double-Blind versus Placebo, Moxifloxacin-Controlled Study

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## Abstract

**Background and Objective:** Prulifloxacin, a broad-spectrum fluoroquinolone, is quantitatively transformed after oral administration into ulifloxacin, the active metabolite. On the basis of preclinical data suggesting that prulifloxacin is not likely to prolong the QT interval, a trial to assess the potential effects of prulifloxacin on QT and corrected QT (QTc) interval in humans was performed.

**Methods:** Fifty-two healthy subjects were randomized into three groups to receive prulifloxacin 600 mg, moxifloxacin 400 mg and placebo once daily for 5 days, using a crossover, double-blind versus placebo, moxifloxacin-controlled study. At baseline and days 1 and 5, three 12-lead digital ECGs were recorded before and up to 24 hours after dosing at nine predefined timepoints. Blood samples were also collected at each treatment timepoint. ECG data were analysed in a blinded manner by a centralized laboratory using skilled readers. QT values were corrected for heart rate using an individual correction method (QTcI) as the primary variable, and Fridericia's method as reference.

**Results:** In forty-eight subjects who completed the study, compared with placebo, prulifloxacin had no relevant effect on cardiac repolarization, with the largest mean QTcI increase being 3.97 ms (one-sided 95% CI 0.01, 7.93), whereas moxifloxacin demonstrated the expected positive effect (maximum mean QTcI increase of 12.0 ms, one-sided 95% CI 8.66, 15.34), thus demonstrating the good sensitivity of the study. A statistically significant correlation between QTcI changes and plasma concentrations was found for moxifloxacin but not for ulifloxacin.

**Conclusion:** Prulifloxacin at steady state after therapeutic doses has no significant effects on the QTc interval and thus should prove to have no cardiac liability.

## Background

Cardiac repolarization is defined as the duration of the corrected QT (QTc) interval on the standard 12-lead ECG, which is determined by a balance between a number of inward and outward cellular ion currents.[1] QTc interval prolongation, usually in conjunction with other risk

factors (e.g. hypokalaemia, atrial fibrillation, congestive heart failure, etc.), can degenerate into torsades de pointes (TdP), a frequently fatal form of polymorphic ventricular tachycardia [1,2]

From a regulatory point of view, prolongation of the QTc interval is currently considered the best available predictor of cardiac safety risk, although still overall an imperfect biomarker [2-4] Indeed, in addition to its low specificity for predicting arrhythmias, other issues relevant to use of the QT interval as a biomarker for cardiac safety risk include an apparent dissociation between QT/QTc interval prolongation and TdP risk for some drugs (e.g. amiodarone, pentobarbital [phenobarbitone] and ranolazine), and the lack of clarity concerning what determines the relationship between QTc prolongation and TdP risk for an individual drug.[3]

Many non-cardiovascular medications have been associated with QT/QTc prolongation.[1] Some fluoroquinolone antibacterial agents have shown an increased risk for QTc interval prolongation (e.g. gemifloxacin and moxifloxacin) in patients with known risk factors who are receiving these drugs, or even have been withdrawn from the market (e.g. sparfloxacin and grepafloxacin) because of this risk.[5]

Substantial differences in the potential for QTc prolongation have been observed among fluoroquinolones, most probably because of their different affinities for the potassium channel. Sparfloxacin and grepafloxacin have been found to be the most potent inhibitors of the *human ether-a-go-go-related* gene (hERG) channel current, moxifloxacin and gatifloxacin have an intermediate inhibitory ability, while levofloxacin and ciprofloxacin have the least inhibitory effects.[6] Although it has been hypothesized that structural differences at position 5 of the quinolone nucleus may affect cardiotoxicity, a structural moiety capable of increasing a drug's potential for QTc prolongation has not yet been identified.[5]

Prulifloxacin is a new broad-spectrum oral fluoroquinolone approved in several European countries for the treatment of patients with acute exacerbations of chronic bronchitis and complicated or uncomplicated lower urinary tract infections at a dosage of 600 mg once daily [7-9] It is characterized by good activity against Gram-positive strains, but particularly against Gram-negative strains, including powerful activity against *Pseudomonas aeruginosa* [10]

After oral administration, prulifloxacin is rapidly absorbed and quantitatively transformed by esterases into ulifloxacin, the active metabolite.[11] Following a single oral dose of prulifloxacin 600 mg, ulifloxacin reaches the maximum plasma concentration ($C_{max}$) of 1.6 μg/mL after 1 hour, with an area under the plasma concentration-time curve (AUC) from time zero to infinity ($AUC_{\infty}$) of 7.3 μg • h/mL.[12] The elimination half-life is approximately 10 hours, allowing once-daily administration.[12]

Prulifloxacin has been assessed for cardiac risk both *in vitro* on the hERG channel and *in vivo* in the conscious dog.[13] Ulifloxacin produced only minor reductions in the hERG current amplitude, lower than those observed with ciprofloxacin, and had no effect on the QTc interval following 5 days of repeated oral administration in the conscious dog monitored by telemetry. In addition, in a rabbit model of arrhythmia, infusions of ulifloxacin (4 mg/kg/min) did not elicit remarkable prolongations in the QT interval, and none of the animals infused with the agent developed arrhythmia.[14] These findings suggested that prulifloxacin is not likely to prolong the QT interval in man.

However, there is no full consensus regarding the predictive value of negative non-clinical studies and the extent to which they can exclude a clinical risk.[4] Therefore, a study to better define the potential cardiac risk associated with administration of prulifloxacin was conducted.

## Subjects and Methods

The study protocol and all other relevant documentation, including the informed consent form, were approved by the Comitato Etico Cantonale of Canton Ticino (Switzerland) on 16 November 2006. The study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice, the Declaration of Helsinki and subsequent amendments, and the European Guidelines for informed consent and protection of study subject rights. Before enrolment, written

informed consent was obtained from each subject who participated in the trial.

### Subjects and Study Design

A total of 48 fully evaluable healthy subjects completed this randomized, three-period crossover, double-blind versus placebo and moxifloxacin-controlled clinical trial.

The primary study parameter was assessment of QTc by 12-lead ECG recordings. Secondary aims of the study were: (i) to evaluate the pharmacokinetic profile of ulifloxacin following single and repeated administration of prulifloxacin; (ii) to assess T-wave morphology and appearance of U waves; and (iii) to monitor the safety profile of study medications by means of vital signs (blood pressure, heart rate), routine blood laboratory parameters and adverse event (AE) recordings.

Participants were male and female Caucasian subjects aged between 19 and 72 years, with a bodyweight within 20% of the ideal height/frame size as reported in the Metropolitan Life Height and Weight Tables, and systolic and diastolic blood pressure (measured after 5 minutes of rest in the supine position) within the range 100–160 mmHg and 50–95 mmHg, respectively. Subjects were excluded if they: had a history or clinical evidence of additional risk factors for TdP, such as heart failure, hypokalaemia, family history of long QT syndrome, or a baseline QTc interval >450 ms; had blood electrolyte values (sodium, potassium, calcium, magnesium, chloride) out of the normal range; or were using concomitant medications, in particular drugs known to prolong the QTc interval.

The clinical part of the study was performed at Cross Research SA Phase I Unit, Arzo, Switzerland, and the centralized ECG assessments were carried out by eResearchTechnology, Inc., Peterborough, UK.

At screening, subjects were informed about the aims, procedures and possible risks of the study, and were evaluated by medical history, physical examination, ECG and clinical laboratory assays (blood and urine). Enrolled subjects were confined at the clinical facility and kept under dietary control, starting from the morning of the day before dosing (day 0) and continuing until the day after the last dosing (day 6).

The test medications (prulifloxacin 600 mg tablets, moxifloxacin 400 mg tablets, and prulifloxacin matching placebo tablets) were administered orally in the morning (at about 8:00am) on a once-daily basis for 5 days (from day 1 to day 5) in fasting conditions. Two 7-day washout intervals elapsed between the three crossover treatment periods. Due to manufacturing problems, moxifloxacin was not masked and was administered in open-label conditions.

Assessment of the effect of prulifloxacin on the QT/QTc interval using the oral therapeutic dose at steady state was considered reasonable to confirm the reassuring preclinical data, including results observed in a rabbit model of arrhythmia in which prulifloxacin was safely administered by a 4 mg/kg/min infusion.[13,14] The steady-state condition was also chosen in order to avoid non-homogeneous drug exposure between subjects, as previously observed after single administrations of prulifloxacin dosages higher than 600 mg.[15]

### ECG Evaluations

On day 0 (baseline), ECGs were recorded in the supine position starting from 8:00am at 0.5, 1, 1.5, 2, 2.5, 3, 4, 8 and 12 hours. On day 1 (single dose) and day 5 (steady state), ECGs were recorded immediately before dosing (which occurred about 8:00am), and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, 12 and 24 hours after dosing. ECG recordings were performed using standard 12-lead equipment (ELI-200/250, Mortara Instrument Inc., Milwaukee, WI, USA) with the capacity for digital signal processing, adequately serviced and calibrated. The operator always applied the same technique regarding skin preparation, lead placement and timing of recording. In order to minimize the potential occurrence of hysteresis, ECGs were performed in stable heart rate conditions (subjects at rest in the supine position, limited physical activity), with three 10-second ECG recordings at 2-minute intervals repeated at each timepoint. For each ECG, QT duration was measured using computer-assisted caliper placement on three consecutive beats in lead II. An alternative lead was

selected if lead II was not acceptable for measurement. Data were transmitted via modem to the centralized ECG laboratory to be assessed by experienced cardiac safety specialists, blinded to time, treatment and subject identity. These specialists were responsible for reviewing all ECGs for correct lead and beat selection and verifying correct caliper placement.

The following main parameters were assessed: RR interval (heart rate), QT interval, PR interval, QRS interval and appearance of U waves. QT values were corrected for heart rate to obtain the QTc interval, using the individual correction formula $QTcI = QT/(RR)^{\beta i}$, where $\beta i$ is the slope of the linear regression between the log-transformed QT values and the log-transformed RR values collected at baseline and on placebo treatment for each subject.[16] Calculations involved application of the regression analysis technique to all individual pretreatment and on-placebo QT and RR intervals as the primary parameter,[17] with use of Fridericia's correction formula ($QTcF = QT/RR^{0.33}$) as a historical control (reference). Tracings were immediately assessed to verify the occurrence of QTc prolongation greater than 500 ms (defined as a specific outlier) using the QTcF formula. In such cases, and after confirmation in a subsequent ECG repeated 15 minutes later, the protocol required that the subject withdraw from the study.

### Plasma Sampling and Assays

After each ECG recording, one 3 mL blood sample was collected from the cubital vein. Blood samples were also collected before dosing on the morning of days 3 and 4.

Each blood sample was collected from an indwelling catheter into heparinized test tubes. Blood samples were immediately centrifuged at 4°C at about 2000 *g* for 10 minutes. The supernatant plasma was separated, divided in two aliquots of at least 0.5 mL each, and transferred into plastic tubes stoppered (airtight) and frozen at −20°C or colder in an upright position until analysis. The plasma vials were unequivocally identified as P1 or P2; the label also reported the number of the subject, the sampling time (hour), the study day and the study period.

The two aliquots stored in dry ice were sent separately to Angelini Pharmaceuticals, ACRAF SpA, Rome, Italy, for measurement of ulifloxacin and moxifloxacin plasma concentrations by prestudy validated high-performance liquid chromatography (HPLC) bioanalytical methods that had been specifically calibrated (ranges 0.025–2.5 μg/mL free base ulifloxacin, and 0.025–5 μg/mL free base moxifloxacin). The performance of the method during routine analysis was monitored using quality control samples. The precision and accuracy of quality controls met the international acceptance criteria, i.e. 100 ± 15% (100 ± 20% at the lower limit of quantification [LLQ]) for accuracy, and relative standard deviation ≤15% (≤20% at LLQ) for precision. No calibration standard was rejected and a correlation coefficient ($r^2$) > 0.99 was obtained for all calibration curves. Kinetica™ (version 4.4.1, Thermo Electron Corporation, Waltham, MA, USA) software was used, according to a non-compartmental pharmacokinetic model, to determine the following parameters: $C_{max}$, time to reach $C_{max}$ ($t_{max}$), and AUC from time zero to time of last measured concentration (24 hours) [$AUC_{24}$].

### Statistical Methods and Sample Size

The following statistical analyses were applied to individual (QTcI) and Fridericia's (QTcF) corrections obtained after single and repeated administrations.

For each of the three ECGs assessed at each timepoint, QT and RR intervals were measured on three consecutive beats, and the correction for the heart rate was applied for each beat. These three QTc values were then averaged to obtain one QTc value for every ECG recording. The three QTc values obtained from each ECG were averaged to obtain a single QTc value for each timepoint and adjusted by subtracting the value measured at time 0 on the same day. Finally, for each timepoint, the single QTc value obtained at baseline was subtracted from the relevant single QTc value obtained during treatment (baseline-subtracted difference).

The baseline-subtracted value was analysed by ANOVA that included sequence, subject within

sequence, period and treatment as sources of variations. Pairwise comparisons were performed using linear contrast as follows: moxifloxacin 400 mg versus placebo, and prulifloxacin 600 mg versus placebo.

The residual error of the ANOVA was used to estimate the one-sided 95% CI of the mean difference (baseline-subtracted) versus placebo. In addition, categorical analyses using different signals were performed. Specific outliers were defined as subjects who had a prolongation of the absolute QTc interval >500 ms during therapy, a change from baseline in QTc interval >60 ms, or incidence of new abnormal U-waves. Nonspecific outliers with 30–60 ms change from baseline in the absolute QTc interval were also identified.

Single plasma concentration values were correlated and graphically displayed with time-matched QTc values. The pharmacokinetic/pharmacodynamic relationship was also explored by applying a linear mixed-effects model to the QTcI changes versus the plasma concentration (as fixed effect) with subject included in the model as a random effect.

Assuming a largest time-matched QTc mean difference from placebo of 4 ms and a standard deviation of 11 ms for QT variability, a sample size of 43 subjects was determined to be adequate to provide an upper bound of the 95% CI difference between prulifloxacin and placebo lower than 10 ms, with a power of 80%.[16-19] To ensure balance for sex and sequence of treatments, the sample size was increased to 48 subjects.

## Results

### Subject Demographics

Forty-eight subjects who completed the study per protocol were considered for the pharmacodynamic (QT/QTc interval evaluation) and pharmacokinetic assessments. Fifty-two subjects were considered for the safety assessments. Four of these subjects discontinued for personal reasons after 1 (one subject) and 5 days of the first treatment period. Demographic data and other characteristics of the study population are shown in table I. A CONSORT diagram of subject flow through the study is shown in figure 1.

### ECG Findings

The mean RR, PR, QRS and QT intervals measured at baseline were similar across treatment groups. On day 1, the mean heart rate was statistically significantly increased compared with placebo at most observed timepoints after administration of moxifloxacin 400 mg (the largest mean increase was 3.94 beats/min; one-sided 95% CI 1.40, 6.49), while no significant changes were detected after prulifloxacin 600 mg administration. On day 5, no significant changes in heart rate in subjects taking either drug were found. Analysis of PR intervals revealed a significant decrease compared with placebo only after administration of moxifloxacin 400 mg. Indeed, on days 1 and 5, the largest mean decreases in PR interval in subjects taking moxifloxacin were −6.29 ms (one-sided 95% CI −9.63, −2.96) and −5.30 ms (one-sided 95% CI −8.79, −1.81), respectively.

The QRS interval evaluation showed a significant increase with both treatments in comparison with placebo on day 1. The largest mean increase was 3.40 ms (one-sided 95% CI −1.61, 5.19) after moxifloxacin administration, and 2.27 ms (one-sided 95% CI 0.95, 3.39) after administration of prulifloxacin.

As described in the Methods section, the relationship between heart rate and QT was accounted

**Table I.** Baseline demographic characteristics of the study population[a]

| Demographic | Value |
|---|---|
| Sex [n] | |
| male | 26 |
| female | 26 |
| Race [n] | |
| Caucasian | 52 |
| Age [y] | 41.6 ± 16.9 (18–72) |
| Bodyweight [kg] | 66.0 ± 9.4 (45–84) |
| Body mass index [kg/m$^2$] | 23.1 ± 2.1 (18.5–25.9) |
| Systolic blood pressure [mmHg] | 119.5 ± 10.3 (100–138) |
| Diastolic blood pressure [mmHg] | 74.7 ± 9.1 (60–94) |

a Values are given as mean ± SD (range) unless otherwise specified.

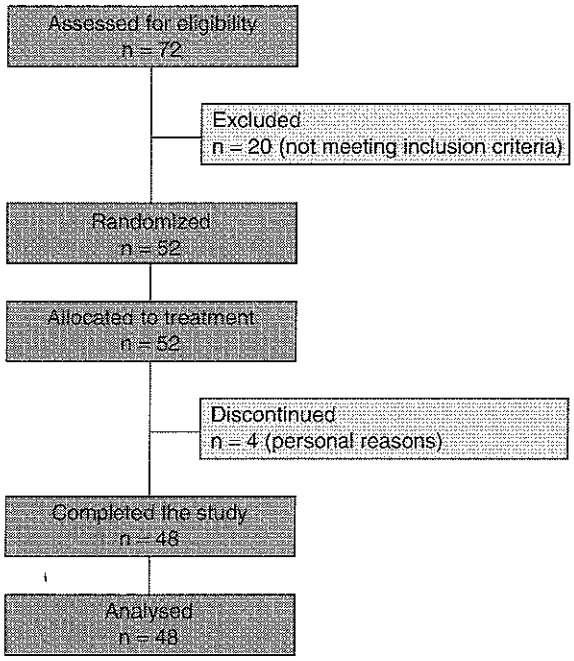


**Fig. 1.** CONSORT diagram of flow of subjects through the study

for by calculating heart-rate-corrected QT intervals according to Fridericia's (QTcF) correction and an individual correction method obtained applying the formula $QTcI = QT/(RR)^{\beta i}$.

The comparisons of the QTcI with prulifloxacin or moxifloxacin versus placebo (baseline-subtracted and predose adjusted) at each timepoint and at the individual $t_{max}$ values are shown in table II. On day 1, the largest mean QTcI increases with prulifloxacin and moxifloxacin compared with placebo were 2.20 ms (one-sided 95% CI −2.20, 6.60 at 24 hours after dosing) and 12.00 ms (one-sided 95% CI 8.66, 15.34 at 1.5 hours after dosing), respectively. On day 5, the largest mean QTcI increases with prulifloxacin and moxifloxacin compared with placebo were 3.97 ms (one-sided 95% CI 0.01, 7.93 at 3 hours after dosing) and 11.74 ms (one-sided 95% CI 8.49, 14.99 at 1 hour after dosing), respectively. Analogous results were observed when the QTcI changes were analysed at the individual $t_{max}$ values (table II). Similar results were observed when using Fridericia's correction.

Figure 2 shows the placebo-corrected, time-matched and predose-adjusted QTcI changes (delta-delta values) after single and repeated administration of prulifloxacin and moxifloxacin.

No specific outliers, as defined in the Methods section, were detected. Some nonspecific outliers with QTc changes of 30–60 ms were detected after single (one with placebo, one with prulifloxacin and five with moxifloxacin) and repeated (one with placebo, three with prulifloxacin and seven with moxifloxacin) administrations.

## Pharmacokinetic Parameters

The $C_{max}$ (mean ± SD) for ulifloxacin after both single and repeated administrations was 1.3 ± 0.5 μg/mL at a median $t_{max}$ of 1.2 hours. The

**Table II.** Mean QTcI differences from baseline, placebo-corrected at each timepoint and at individual time to reach maximum plasma concentration ($t_{max}$)

| Time [h] | Mean QTcI differences vs placebo (one-sided 95% CI) [ms] | | | |
|---|---|---|---|---|
| | prulifloxacin | | moxifloxacin | |
| | day 1 | day 5 | day 1 | day 5 |
| 0.5 | −3.69 (−6.78, 0.60) | 0.75 (−2.21, 3.70) | 4.29 (1.20, 7.37) | 7.27 (4.32, 10.22) |
| 1 | 1.24 (−2.01, 4.50) | 2.57 (−0.68, 5.82) | 10.12 (6.86, 13.37) | 11.74 (8.49, 14.99) |
| 1.5 | 1.31 (−2.03, 4.65) | 0.45 (−2.88, 3.78) | 12.00 (8.66, 15.34) | 8.11 (4.78, 11.43) |
| 2 | 1.92 (−1.43, 5.27) | 2.35 (−1.09, 5.79) | 9.63 (6.28, 12.98) | 8.67 (5.23, 12.11) |
| 2.5 | 1.77 (−1.79, 5.34) | 1.13 (−2.09, 4.35) | 11.45 (7.89, 15.01) | 9.15 (5.93, 12.37) |
| 3 | 1.83 (−1.80, 5.46) | 3.97 (0.01, 7.93) | 10.95 (7.32, 14.58) | 8.43 (4.47, 12.38) |
| 4 | 1.37 (−2.24, 4.98) | 3.35 (−0.38, 7.08) | 7.25 (3.64, 10.86) | 7.72 (3.99, 11.45) |
| 8 | 0.26 (−3.35, 3.87) | 2.99 (−0.70, 6.68) | 4.05 (0.43, 7.66) | 6.38 (2.69, 10.07) |
| 12 | −0.23 (−3.87, 3.41) | 1.54 (−2.43, 5.52) | 5.51 (1.86, 9.15) | 6.11 (2.14, 10.08) |
| 24 | 2.20 (−2.20, 6.60) | 1.86 (−1.73, 5.46) | 4.85 (0.45, 9.25) | 2.46 (−1.14, 6.05) |
| Individual $t_{max}$ | 0.30 (−3.21, 3.81) | 1.93 (−1.37, 5.22) | 9.52 (6.35, 12.69) | 10.32 (6.69, 13.95) |

**QTcI** = QT value corrected for heart rate using an individual correction method.

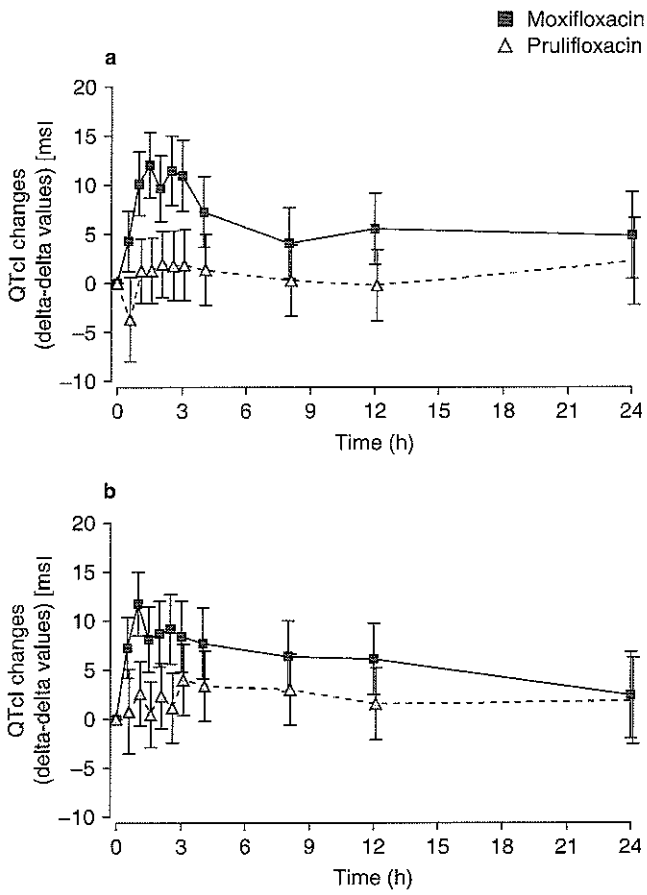


**Fig. 2.** Placebo-corrected, time-matched and predose-adjusted QT values corrected for heart rate using an individual correction method (QTcI) as primary variable (delta-delta values) [mean and one-sided 95% CI] after (**a**) single and (**b**) repeated administration of prulifloxacin and moxifloxacin

$AUC_{24}$ values (mean ± SD) for ulifloxacin were 6.1 ± 2.3 μg • h/mL and 6.7 ± 2.3 μg • h/mL following single and repeated administrations, respectively. The $C_{max}$ (mean ± SD) values for moxifloxacin after single and repeated administrations were 3.5 ± 0.9 μg/mL and 4.1 ± 0.9 μg/mL at a median $t_{max}$ of 3.3 and 4.1 hours, respectively. The $AUC_{24}$ values (mean ± SD) for moxifloxacin were 34.5 ± 8.4 μg • h/mL and 46.6 ± 10.2 μg • h/mL following single and repeated administrations, respectively.

### Pharmacokinetic/Pharmacodynamic Analysis

The pharmacokinetic/pharmacodynamic relationship between ulifloxacin or moxifloxacin plasma concentration and the QT interval was investigated in pooled data by linear regression and linear mixed-effects models. The analysis, including placebo data, was applied to the QTcI baseline-subtracted difference and predose adjusted values, calculated at each timepoint. Regression analysis showed no correlation between QTcI changes and ulifloxacin concentrations ($r = 0.01064$, $p < 0.69$), while a statistically significant correlation between QTcI changes and moxifloxacin concentrations ($r = 0.39967$, $p < 0.0001$) was detected (figure 3). The linear mixed-effects model confirmed a statistically significant relationship between QTcI changes and moxifloxacin plasma concentration ($p < 0.0001$), but no statistically significant relationship between QTcI changes and ulifloxacin plasma concentration ($p = 0.0720$).

### Safety and Tolerability

Fifty-two randomized subjects (48 completers plus four drop-outs) were included in the safety analysis.

Fifty AEs of mild or moderate intensity occurred during the study: 13 with prulifloxacin, 28 with moxifloxacin and nine with placebo. All patients with AEs recovered fully without sequelae. The numbers of AEs judged as probably or possibly treatment related were 11/13 with prulifloxacin, 20/28 with moxifloxacin and 2/9 with placebo. The most frequently reported treatment-related AEs during prulifloxacin and moxifloxacin treatment were headache and nausea, respectively. No serious AEs occurred throughout the study.

Some minor or not clinically significant deviations from normal ranges in laboratory parameters were observed; these were homogeneously distributed among treatments. Statistical analysis of vital signs showed a significant decrease in diastolic blood pressure in the placebo group. These variations appeared to be randomly distributed and were judged to be not clinically significant.

## Discussion

This study was designed to investigate the potential cardiotoxic effect of ulifloxacin, the active metabolite of prulifloxacin [7,17]. The study enrolled

48 evaluable healthy subjects treated according to a crossover design in double-blind conditions with repeated standard dosages of prulifloxacin placebo and moxiflaxacin Due to manufacturing problems, the positive control moxifloxacin was not masked. The crossover design included a 7-day washout period, allowing subsequent treatment courses to be unaffected by prior courses, as demonstrated by baseline QT/QTc and pharmacokinetic data.

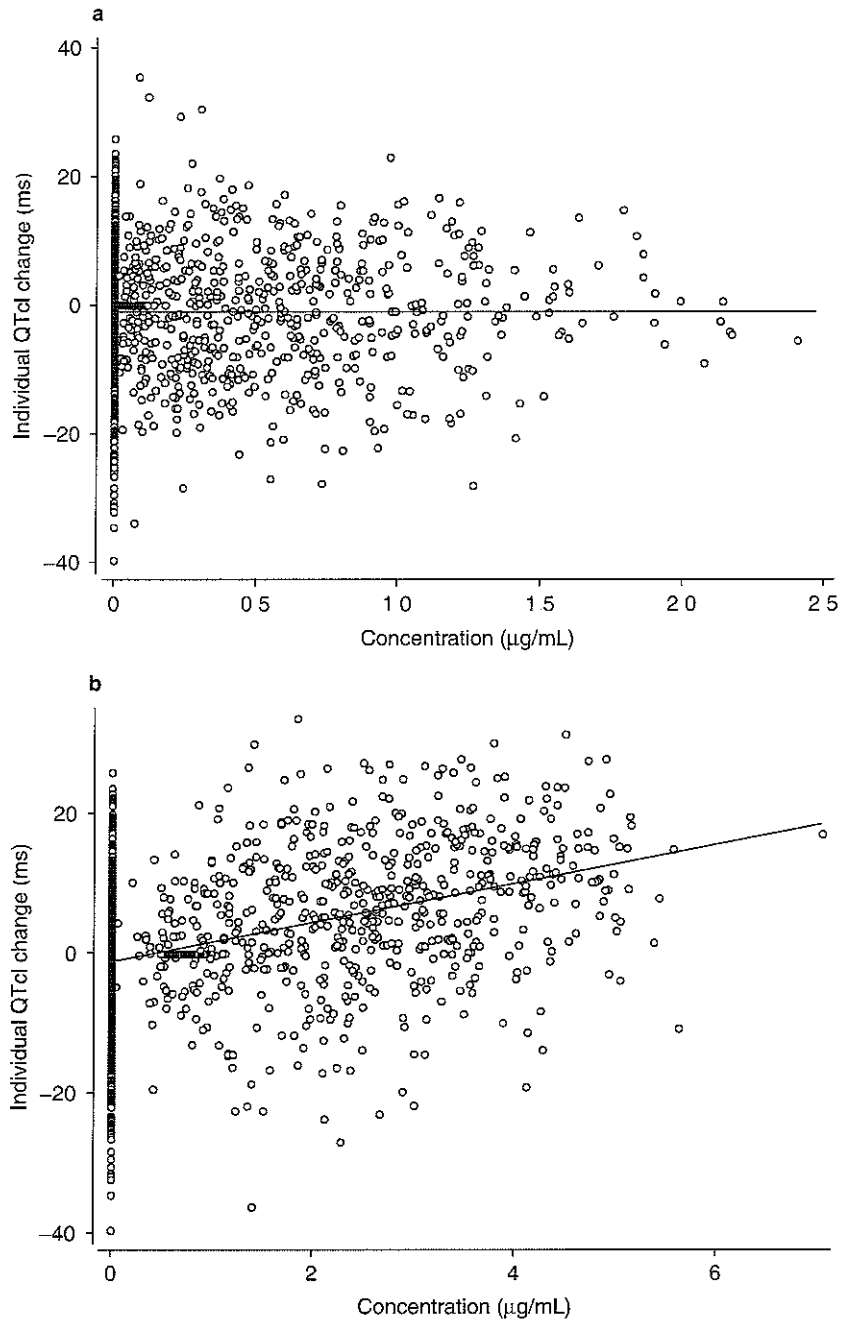


**Fig. 3.** Regression analysis of QT values corrected for heart rate using an individual correction method (QTcI) as primary variable (delta values) vs plasma concentration of (**a**) ulifloxacin/placebo and (**b**) moxifloxacin/placebo; data for both figures are pooled data from days 1 and 5. No correlation between QTcI changes and ulifloxacin concentration was detected ($r=0.01064$, $p<0.69$) whereas there was a statistically significant correlation between QTcI changes and moxifloxacin concentration ($r=0.39967$, $p<0.0001$)

The 5-day repeated dosage regimen was chosen to achieve steady state, to replicate the normal use of the drugs, and to investigate the possible appearance of pharmacodynamic tolerance [20]

The accuracy of the ECG assessments, and the correctness of the study procedures in general, allowed us to obtain very good sensitivity levels for the methodology applied; this was confirmed by the results observed in subjects treated with moxifloxacin, a widely used positive control. Indeed, the known effect of moxifloxacin of prolonging the QT interval was observed throughout the study, regardless of the correction method used, and our findings in regard to this effect of moxifloxacin compare well with previous data reported in the literature.[4,19,21] The largest QTcI increase after moxifloxacin administration compared with placebo was approximately 12.00 ms (one-sided 95% CI lower limit of 8.66 ms). Thus, assay sensitivity in the trial was established.[18] Conversely, prulifloxacin administration did not produce QTcI prolongations longer than 4 ms at any timepoint, and the 10 ms 95% CI upper limit was never breached. The prulifloxacin effect fell within the interval 0–5 ms, which is considered in the literature to be a range with no risk for TdP.[2] The largest QTcI mean increase compared with placebo during prulifloxacin administration was 3.97 ms (one-sided 95% CI 0.01, 7.93), reported at day 5 at 3 hours after dosing.

In the pharmacokinetic/pharmacodynamic analysis, no correlation between QTcI changes and ulifloxacin plasma concentrations was detectable, while a statistically significant correlation between QTcI changes and moxifloxacin plasma concentrations was found. In addition, no prolongation of the absolute QTc interval >500 ms, no changes from baseline in absolute QTc interval >60 ms, and no new abnormal U-waves were found.

According to the above data, this trial demonstrated that prulifloxacin at steady state after therapeutic doses has no potential effect on cardiac repolarization measured by QT/QTc interval prolongation. However, because the trial involved healthy subjects and used conventional repeated dosages of prulifloxacin, its results cannot be extrapolated to patients at high risk for TdP. These aspects may represent a limitation of the study, even if the QTc interval measurement is considered an imperfect biomarker for cardiac safety and appears to have low specificity for predicting arrhythmias.[2-4] Thus, data reported in this article cannot be considered to represent a definitive assessment of the cardiac safety profile of prulifloxacin. However, they do provide an important piece of information to be assessed in conjunction with negative cardiac risk findings observed in non-clinical *in vitro* and *in vivo* studies[13,14] and post-marketing surveillance data.[15] In this context, prulifloxacin can reasonably be considered to be a drug with a positive risk/benefit ratio in terms of its potential for cardiotoxic effects.

## Conclusion

This study suggests that there should be minimal, if any, risk of cardiac-related events with clinical use of prulifloxacin at steady state after therapeutic doses. Accordingly, the potential for new indications for prulifloxacin can be investigated in appropriate clinical trials without the need for intensive monitoring of the QTc interval.

## Acknowledgements

This work was supported by a grant from Angelini Pharmaceuticals, ACRAF SpA, Rome, Italy. The authors are employees of Angelini Pharmaceuticals. The authors would like to thank Dr Joel Morganroth for his invaluable editorial assistance.

## References

1. Shah RR. Drug-induced prolongation of the QT interval: why the regulatory concern? Fundam Clin Pharmacol 2002; 16 (2): 119-24
2. Morganroth J. Cardiac repolarization and the safety of new drugs defined by electrocardiography. Clin Pharmacol Ther 2007; 81 (1): 108-13
3. Sager PT. Key clinical considerations for demonstrating the utility of preclinical models to predict clinical drug-induced torsades de pointes. Br J Pharmacol 2008; 154 (7): 1544-9
4. Shah RR. Drugs, QTc interval prolongation and final ICH E14 guideline: an important milestone with challenges ahead. Drug Saf 2005; 28 (11): 1009-28
5. Mehlhorn AJ, Brown DA. Safety concerns with fluoroquinolones. Ann Pharmacother 2007; 41: 1859-66
6. Kang J, Wang L, Chen XL, et al. Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K+ channel HERG. Mol Pharmacol 2001; 59 (1): 122-6

7. Keam SJ, Perry CM. Prulifloxacin. Drugs 2004; 64 (19): 2221-34
8. Cazzola M, Salvatori E, Dionisio P, et al. Prulifloxacin: a new fluoroquinolone for the treatment of acute exacerbation of chronic bronchitis. Pulm Pharmacol Ther 2006; 19 Suppl 1: 30-7
9. Carmignani G, De Rose AF, Olivieri L, et al. Prulifloxacin versus ciprofloxacin in the treatment of adults with complicated urinary tract infections. Urol Int 2005; 74 (4): 326-31
10. Roveta S, Schito AM, Marchese A, et al. Microbiological rationale for the utilisation of prulifloxacin, a new fluoroquinolone, in the eradication of serious infections caused by *Pseudomonas aeruginosa*. Int J Antimicrob Agents 2005; 26 (5): 366-72
11. Tougou K, Nakamura A, Watanabe S, et al. Paraxonase has a major role in the hydrolysis of prulifloxacin (NM441), a prodrug of a new antibacterial agent. Drug Metab Dispos 1998; 26 (4): 355-9
12. Picollo R, Brion N, Gualano V, et al. Pharmacokinetics and tolerability of prulifloxacin after single oral administration. Arzneimittelforschung 2003; 53 (3): 201-5
13. Lacroix P, Crumb WJ, Durando L, et al. Prulifloxacin: in vitro (HERG current) and in vivo (conscious dog) assessment of cardiac risk. Eur J Pharmacol 2003 Sep 5; 477 (1): 69-72
14. Akita M, Shibazaki Y, Izumi M, et al. Comparative assessment of prulifloxacin, sparfloxacin, gatifloxacin and levofloxacin in the rabbit model of proarrhythmia. J Toxicol Sci 2004; 29 (1): 63-71
15. Data on file, ACRAF SpA, Rome, 2009
16. Malik M, Camm AJ. Evaluation of drug-induced QT interval prolongation: implications for drug approval and labelling. Drug Saf 2001; 24 (5): 323-51
17. Committee for Medicinal Products for Human Use (ICH Topic E14). Note for guidance on the clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. CHMP/ICH/2/04 London, 25 May 2005
18. Committee for Medicinal Products for Human Use (ICH Topic E14). The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs: questions and answers. EMEA/CHMP/ICH/310133/2008 June 2008
19. Isikouris JP, Peeters MJ, Cox CD, et al. Effects of three fluoroquinolones on QT analysis after standard treatment courses. Ann Noninvasive Electrocardiol 2006; 11 (1): 52-6
20. Padrini R, Gusella M, Al Bunni M, et al. Tolerance to the repolarization effects of rac-sotalol during long-term treatment. Br J Clin Pharmacol 1997; 44 (5): 463-70
21. Kubitza D, Mueck W, Becka M. Randomized, double-blind, crossover study to investigate the effect of rivaroxaban on QT-interval prolongation. Drug Saf 2008; 31 (1): 67-77

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